STUDIES ON THE CELLS OF CATTLE WITH SPECIAL REFERENCE TO SPERMATOGENESIS, OÖGONIA, AND SEX-DETERMINATION.

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CONTENTS.

I. INTRODUCTION.

Sex-determination with its many attendant problems has always been a subject of great interest to practical animal breeders; and the art of breeding has always been replete with rules by which the sex ratio might be shifted in various ways to the advantage of the breeder. These rules, however, have been founded upon inadequate evidence and unsound reasoning.

The most common beliefs in regard to sex control have from time to time been founded on heat relations, some maintaining that the products of conception in early heat were more often males, others that they were more often females. Pearl and Parshley ('13) have published data on sex-determination in cattle with the following conclusions: (1) That as the time of coitus approaches the end of the œstrous period there is a pro-

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gressive increase in the proportion of male young born. (2) That in the extreme case this increase in the proportion of male births is probably statistically significant and not to be attributed to errors of random sampling. (3) That these modifications of the sex ratio cannot be attributed to age differences or to any other factor yet suggested.

More recent and extensive data, however, according to Pearl ('17) make the relation of time of service to sex extremely doubtful. He says: "The apparent relation between these two factors, which is believed by many breeders to exist and which our earlier statistics appeared to indicate, seems now to be purely accidental, and to have arisen only because of the comparative meagerness of the statistics on which the matter was discussed." Sex in cattle is a matter of heredity, as is shown by the results of this investigation, and remains a matter beyond the control of the breeder; its ultimate control is problematical. However, the suggestion of experimentally separating the two types of spermatozoa, or destroying the one type without impairing the nature of the other, does not appear to be entirely hopeless. If this can be done, the problem of sex control will be comparatively simple, since artificial insemination can be resorted to.

It appears to be a well-established fact that sex is determined at the time of fertilization. Sex, like other characters of the individual, has a definite factorial basis; and the factorial constitution of the individual with respect to sex as well as to other characters is fixed by the constitution of the two gametes which unite to form the zygote.

The dimorphic condition among the spermatozoa in many of the lower animal forms is well known. Guyer pointed it out in the rat ('10), and man ('10); other investigators have reported it in several other mammals. Among the domestic mammals the same condition has been clearly demonstrated in the pig (Wodsedalek, '13) and in the horse (Wodsedalek, '14). This study on the sex cells of cattle shows that two types of spermatozoa are also produced in the bull; the one type at the time of fertilization determining maleness and the other type determining femaleness. The spermatozoan which determines femaleness is somewhat

larger owing to the presence of a large sex-chromosome or accessory element which is lacking in the type producing maleness. Since it has been found in this study that the oögonia of cattle possess two sex-chromosomes it is safe to conclude, in view of our knowledge of oögenesis in general, that all of the mature ova carry the reduced number or one sex-chromosome. If the ovum is fertilized by a sperm which lacks the sex-chromosome the resulting zygote naturally possess only one sex-chromosome and, therefore, develops into a male. This is evidenced by the fact that the somatic as well as the germ cells of the male possess a single accessory element. On the other hand, if the ovum is fertilized by a sperm possessing the sex-chromosome the resulting zygote possesses two sex-chromosomes and, therefore, develops into a female. The somatic as well as the germ cells of the female possess the two accessory elements which is in exact accord with expectations.

The results presented in this paper were obtained through a most careful and critical study extending over a period of more than four years. The problem was started in the spring of 1915 and every summer since and a great deal of the spare time in the intervening school years, with the exception of the year 1918, has been devoted to it. In the aggregate this means a total of about fifteen months of continuous work. Practically all of the results presented in this paper were at hand at the end of the first two and a half years of study. Over four hundred pencil sketches were made of the various cells, especially those in mitosis, with the aid of the camera lucida and the location of each, as indicated by the mechanical stage, was carefully recorded in a separate booklet. A brief summary of the results of the study was written up and all of the material was put away for a whole year (1918) during which the summer months and other spare time was devoted to the study of the sex cells in another mammal. No effort was made to remember any of the results of the study on the sex cells in cattle.

In the meantime, considerable new cattle material was obtained and hundreds of new slides were made by the departmental technician. At the end of the year the new cattle material was studied and the results were carefully recorded. The original

material was then reinvestigated and new sketches were made of many of the cells, the location of which was recorded before. The results obtained through the study of new slides prepared by the technician, as well as those obtained through the second study of my original material were then compared with the original results. Several hundred different germinal and somatic cells from both sexes were carefully checked up. Especial attention was paid to the number of the ordinary chromosomes and to the sex-chromosomes. Only in a comparatively few cases was there any discrepancy in the interpretation of the nature and number of the ordinary chromosomes. Conditions pertaining to the sex-chromosomes were corroborated in every instance. I was further checked up on my chromosome counts by several of my assistants and senior pre-medical students who had considerable training in microscopic anatomy. In general, these men who knew nothing of my own interpretations, corroborated my counts in a surprisingly large number of instances.

II. Material and Methods.

All of the material used in this investigation with the exception of some ovaries was obtained through the courtesy of the management of the Hagan and Cushing Packing Plant which adjoins the University farm. Some excellent ovarian tissue was given to me by my colleague Dr. A. R. Hahner, formerly professor of veterinary science of the University of Idaho. The ovaries were removed from two five-months-old heifers of the university herd. Testes were obtained from seven mature bulls and one male fœtus of five months and from six smaller fœtuses varying from two to eight and one half inches in length. The ovaries were obtained from four heifers and as many cows, and from five small fœtuses, varying from two and one half to seven inches in length. In addition to this six small embryos were sectioned. Many slides were also made of various somatic structures from the small fœtuses of both sexes.

Several fixing fluids were tried on the testicular material, including Hermann's, Gilson's, Flemming's, and Bouin's. Bouin's fluid used straight or with slight modifications, at 38° C. was the most universally successful fixing agent. When used at some-

what lower temperatures, the material showed little or no modifications. When Bouin's fluid was modified, the alteration took the form of the addition of a small amount of chromic acid, or urea, or the reduction of the percentage of acetic acid. All of these slight modifications gave very good results in the testicular material, and the ovarian, embryological and fœtal tissues appeared to be best when fixed in the fluid modified with chromic acid. The cold method (Hance '17) was also tried in two instances with the testicular tissue but with less success. And while I have not tried this out on the cattle tissue myself, I have every assurance that the laboratory technician carried out the process with great care.

In the study of the male germ cells, smears as well as sections were used. Many stains and counter stains were tried. Iron-hæmatoxylin when used alone was found to be the most satisfactory. All of the figures represented in this paper were made from material stained in this manner.

III. General Arrangement of the Male Germinal Cells.

The structure of the testes of the bull is similar to that of the other well-known mammals and bears a great resemblance to the conditions found in the testes of the horse (Wodsedalek '14). The interstitial cells as in the horse are small and fewer in number in comparison with their large size and great abundance in the testes of the pig. The size of the seminiferous tubules, as well as the general size of the various germinal cells, however, corresponds to the condition found in the pig. The usual types of cells, (1) spermatogonia, (2) primary spermatocytes, (3) secondary spermatocytes, (4) spermatids, and (5) spermatozoa in various stages of development, are present in great abundance.

IV. Spermatogenesis.

In general the spermatogenesis of the bull corresponds to that of the pig and the horse. Since many of the finer cytological points are given in detail in the papers on the pig and the horse (Wodsedalek '13 and '14) they are omitted here to avoid unnecessary duplication. And while all of the finer details involved in a thorough piece of work in spermatogenesis were carefully

studied in this animal, only the phases pretaining to the chromosome numbers and their behavior are emphasized in this paper.

1. *Spermatogonia.*

The spermatogonia usually lie in a single layer next to the wall of the tubule, though occasionally some of the cells are crowded out, thus forming a second layer which is always very irregular. The cells which undergo the last spermatogonial division (Figs. 5–12) are usually beyond the first layer, though occasionally they may be found next to the tubule wall along the entire section of the tubule. At times the cells are far apart, in which case they are flattened out on the tubule wall. The cells also differ considerably in size and appearance, depending on the stage of development they are in.

During the resting stage a large nucleolus is invariably present. As a rule it assumes a somewhat heart-shaped appearance; especially is this true in the larger cells and in those in which the chromosomes begin to form. At the conclusion of the resting stage numerous large chromatin granules appear and arrange themselves along fine threads in an entangled mass. The chromosomes soon become distinct and mitotic figures are fairly numerous. And while, as a rule, there is considerable overlapping and massing of the chromosomes in the early spermatogonial divisions, hundreds of cells were found in which there was little or no overlapping, making accurate counts possible.

Thirty-seven chromosomes appear in the late prophase of the spermatogonial division (Fig. 1). Thirty-six of these are variously shaped, mainly oblong or slightly curved, and differ somewhat in size. One which is much larger is triangular in form or heart-shaped. This is the accessory or sex-chromosome, and is the same thing as the large nucleolus which appears in the resting stages. This point is certain, as the body can be easily traced through the various stages of the cells. A similar condition was reported by Guyer ('10) in man, Wodsedalek in the pig ('13) and in the horse ('14). Several other investigators have reported it in other forms since. During division each chromosome, including the sex-chromosome, divides in two (Figs. 3 and 4).

In the last spermatogonial cells the chromosomes appear in a dense mass. The cells gradually increase in size and great expansion takes place in the nuclei (Figs. 5–9). The chromosomes become decidedly distinct and surprisingly well segregated throughout the spherical nucleus (Figs. 10 and 11). The cells in these stages are numerous and beautiful. The chromosomes appear to be dense in structure and are thicker than those of the early spermatogonial cells. Several of the chromosomes are almost spherical. They are so evenly distributed that hundreds of accurate counts can be made within a short time. This condition prevailed in all of the mature testes studied and the cells were especially numerous in three two-year-old bulls. Even in somewhat stale tissue (Fig. 12) the chromosomes in these cells appear to remain well segregated. Altogether over one thousand accurate counts were made in these cells alone.

2. *Primary Spermatocytes.*

The sex-chromosome can invariably be seen in the spermatocytes where it retains its individuality (Fig. 13). Just how pairing takes place in these cells cannot be stated with certainty. When the chromosomes appear for division they are of the reduced number and bivalent in nature. The thirty-six ordinary chromosomes pair while the sex-chromosome remains unpaired and can easily be distinguished from the others (Figs. 14–18). It occasionally shows its double nature in the late prophase (Fig. 17), and more frequently in the later stages of the primary spermatocyte division (Figs. 22, 24, 25, 27, 28 and 29).

During the primary spermatocyte division the sex-chromosome usually passes to one pole in advance of the other chromosomes (Figs. 19–25). This unequal division of the chromosomes in these cells (Figs. 19–29) gives rise to two different types of secondary spermatocytes. The one type containing the eighteen ordinary chromosomes plus the sex-chromosome. Just before division is complete the chromosomes become loosely paired (Figs. 28 and 29). This peculiar behavior of the chromosomes is apparently quite common in mammalian tissue.

3. *Secondary Spermatocytes.*

No resting stage occurs in the secondary spermatocyte. This condition also occurs in the horse and is frequently found in the pig, according to my former studies. The secondary spermatocytes divide soon after they are formed and not infrequently the spindles are formed in the two cells resulting from the first spermatocyte division while they are still in close contact. Nine chromosomes arrange themselves in the equatorial plate for division in the one type of secondary spermatocyte (Fig. 31), and nine plus the sex-chromosome in the other (Fig. 30). All of the chromosomes, including the sex-element when it is present, divide in these cells (Figs. 32–38).

4. *Spermatids.*

The division of the secondary spermatocyte gives rise in the one case to spermatids containing nine chromosomes (Figs. 36–38), and in the other case nine plus the sex-chromosome or ten (Figs. 33 and 34). All of the chromosomes except the sex-chromosome are bivalent in nature so that in reality we have the equivalent of eighteen chromosomes in the one kind of spermatid and eighteen plus the sex-chromosome in the other. The bivalent chromosomes frequently begin to separate before the division of the cell is complete. Occasionally the eighteen chromosomes can be distinguished as independent elements after the cell divides (Fig. 38), although the chromosomes usually disintegrate before complete separation can be identified. All of the foregoing evidence indicates that eighteen is the reduced number of chromosomes.

V. Dimorphism in the Spermatozoa.

The spermatozoa of the bull vary considerably in size, and careful measurements show that they may be arranged in two separate classes, one type being much larger than the other. Mature specimens, which were free in the lumen of the tubule and parallel to the objective, were selected at random from a single slide and outline sketches of six hundred heads were made with the aid of a camera lucida. The lengths of the sketches were then carefully measured and recorded in quarter millimeters. Figure 1 in the text shows the variation in size of the six hundred

heads measured. It shows a distinct bimodal curve with modes at 12.50 mm., and 14.75 mm. The intermodal depression is

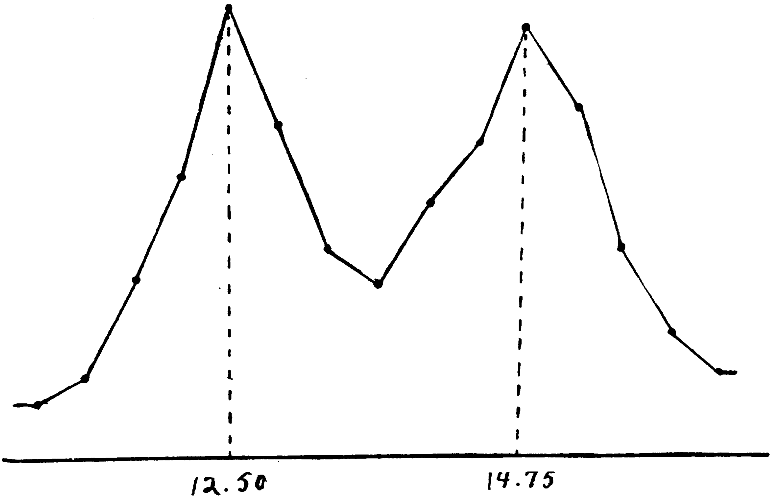

FIG. 1. Diagram showing the variation in size among six hundred mature spermatozoa of the bull.

deep and wide and the two elements of the curve are approximately equal as regards number of individuals. The spermatozoa of the larger type undoubtedly possess the sex-chromosome, while those of the smaller type are without it. Figures 39 and 40 show the comparative size of the two distinct types of mature spermatozoa. They can be distinguished with ease under a high-power microscope.

The general scheme of the development of the spermatozoön from the spermatid of the bull is similar to that in the pig and the horse (Wodsedalek '13 and '14) and, therefore, will not be described here. Bimodal curves were also shown by the writer in connection with the spermatozoa of the pig and the horse, and in a number of other species by Zeleny and Faust ('14 and '15) and by Zeleny and Senay ('15).

VI. Oögonia.

The best ovarian material studied in connection with the number of chromosomes in the oögonia was obtained from some of the small fœtuses. The results obtained from this material

were corroborated in the tissue obtained from cows and heifers, although the adult tissue was not nearly as satisfactory. Most excellent material was found in the ovaries of a four and a half inch fœtus and one six-inch fœtus. These were fixed in the Bouin's plus chromic acid fixing fluid. The oögonia were apparently in extreme activity at the time of fixation. Mitotic figures are abundant and the chromosomes are very distinct, especially in the late prophase and early metaphase stages which are numerous (Figs. 44–46). In general these cells resemble the last spermatogonial cells (compare Figs. 44 and 10), except that the oögonia are somewhat larger.

In well fixed and favorably stained material the oögonia in the resting stage invariably show two large nucleoli each corresponding to the single nucleolus of the resting stage of spermatogonial cells (Fig. 41). These retain their individuality during the spireme stage though at times they are somewhat distended (Fig. 42). When the spireme breaks up the chromosomes are long and narrow and variously curved (Fig. 43). In the late prophase they become shorter and thicker and appear evenly distributed throughout the large nuclei (Figs. 44 and 45). In this stage as well as in the early metaphase stages of division (Fig. 46) hundreds of accurate counts were possible. The two sex-chromosomes can be easily distinguished in all of the prophase stages (Figs. 41–45).

Thirty-eight chromosomes are present in the oögonia (Figs. 43–46). Thirty-six are the ordinary chromosomes corresponding to the thirty-six ordinary chromosomes in the spermatogonia of the male. The two other elements are the sex-chromosomes. When the chromosomes arrange themselves in the equatorial plate for division the sex-chromosomes are always at the periphery (Figs. 46 and 47). During division all of the chromosomes, including the sex-elements, divide in two (Fig. 48). All of the figures of the oögonial cells were made from the same section (Figs. 41–48).

On account of the great significance of the two sex-chromosomes in the femal tissue a tremenduous amount of time was devoted to this particular phase of the problem; this was also true of the studies in connection with the chromosomes of the

somatic tissue in both sexes. The final preparation of the paper for publication was postponed on several occasions, not at all because of any uncertainties, since the results were convincing from the start, but rather because each additional survey of the entire problem from new material proved more fascinating and gratifying than those preceding.

VII. Chromosomes in Somatic Cells.

Numerous slides were made of various somatic structures from fœtuses of both sexes. The organs most frequently used were the brain, lung, liver, Wolffian body, kidney, and intestine. The larger embryos were cut into pieces all of which were then sectioned. Three small embryos, ranging from ten to fourteen millimeter neck-lengths were sectioned in toto. While splendid mitotic figures and late prophase stages were found in many parts of the embryos the very best cells were found in the brain. In the larger specimens the liver and kidneys showed the most favorable cells.

The male somatic cells, like the spermatogonia, contain thirty-seven chromosomes, of which thirty-six are the ordinary chromosomes and one is the accessory element or sex-chromosome (Figs. 51 and 52). The female somatic cells, like the oögonia, contain thirty-eight chromosomes, of which thirty-six are the ordinary chromosomes and the other two are the sex-elements (Figs. 49 and 50). The sex-chromosomes in each case were as distinguishable as they are in the germinal cells. Literally thousands of somatic cells were carefully studied in each sex.

After the chromosomes of the first one hundred of the most favorable male somatic cells were studied and carefully sketched it was found that in ninety-three cases there were thirty-six of the ordinary chromosomes present plus the one accessory. In the other seven cases there were slight discrepancies, usually one or two less. This was in all probability due to unnoticeable overlapping. In two cases there were two extra chromosomes present. This was in all probability due to the fact that two of the chromosomes had divided, since these cells were in the early metaphase stage. This interpretation appears to be correct, since it was found later that occasionally some of the chromo-

somes divide considerably in advance of the others. This point was observed in several of the early metaphase stages; it was fairly common in polar views of late metaphase stages, and in a few instances a chromosome was found almost completely split in two even in the late prophase. For this reason, in selecting cells for accurate chromosome counts great care must be exercised not to select polar views of late metaphase stages. The increase in number may also be due to fragmentation, although one would expect this only in poorly fixed material or general poor technique.

After the counts in the first one hundred camera-lucida sketches showed that over ninety per cent. of the cells contained thirty-seven chromosomes, no more sketches were made. However, hundreds of other counts were made from favorable cells off-hand with about the same results. This shows that in at least ninety per cent. of the male somatic cells thirty-six ordinary chromosomes, and a single sex-chromosome were present. The ten per cent. of discrepancies is undoubtedly due to unnatural states and probable sources of error. The studies of the chromosomes in the somatic cells of the female were conducted in the same manner. Among the first one hundred cells, ninety-one showed thirty-six ordinary chromosomes and two accessories. In several hundred further counts, the percentage of discrepancies was about the same.

In the case of the six small embryos in which sex could not be determined morphologically, or was uncertain, one was poorly fixed, although it was cut into small pieces, and, therefore, could not be used in this study. Of the other five two were unquestionably female and three were male, according to evidences from the cytological standpoint. In the case of the two specimens, cells in various parts of the body repeatedly showed thirty-six ordinary chromosomes and two accessories. In many instances where the ordinary chromosomes could not be counted, the two sex chromosomes were recognizable. In the other three specimens the cells invariably showed only a single sex-chromosome, and in many cases the thirty-six ordinary chromosomes were counted.

These extensive studies indicate quite conclusively that thirty-six is the number of ordinary chromosomes in the somatic cells of both sexes and that the male cells contain one sex-chromosome

while the female cells contain two, making a total of thirty-seven in the male and thirty-eight in the female. These numbers correspond exactly with those of the spermatogonia in the male and the oögonia in the female. This is very significant in relation to our chromosome theory of sex-determination.

VIII. Sex-Chromosomes in Relation to Sex-determination.

It was shown that in the process of spermatogenesis two distinct types of spermatozoa are produced of exactly the same number. The one type contains eighteen ordinary chromosomes plus one sex-chromosome, and the other type contains only the eighteen ordinary chromosomes; this being the result of the unequal primary spermatocyte division, where the eighteen bivalent chromosomes divide and the unpaired sex-chromosome passes over to one pole undivided. In the oögonia there are thirty-six ordinary chromosomes plus two sex-chromosomes. Before the reduction division of the primary oöcytes, in all probability (though this was not actually determined in this animal), all of the chromosomes, including the two sex-chromosomes, pair. This eventually gives rise to ova all of which contain the reduced number of chromosomes or eighteen ordinary chromosomes plus one sex-chromosome.

Since the two types of spermatozoa are produced in equal numbers, fertilization by the one kind or the other is equally possible, and the number of male and female calves born is about equal if a fairly large number of offspring is considered. Sex in the offspring, as determined at the time of fertilization of the ovum by the one or the other type of spermatozoön may be illustrated as follows:

Spermatozoa.		Ova.		Offspring.
$(18 + 1)$	$+$	$(18 + 1)$	$= (36 + 2) =$	female,
$(18 + 0)$	$+$	$(18 + 1)$	$= (36 + 1) =$	male.

The results of the above combinations are in exact accord with the number of chromosomes found in the germinal and somatic cells of the two sexes in cattle; and the relation of the sex-chromosomes to sex-determination can not be doubted. All of the five hundred or more so-called theories or rules for con-

trolling sex, including the one in relation to time of service which is commonly practiced by animal breeders, must be abandoned. There is considerable literature on the metabolic theories of sex-determination which will not be discussed here. However, reference may be made to the brief though able discussion of this subject by Babcock and Clausen ('18).

IX. Sex Ratio in Cattle.

It might be well to quote here Pearl's ('17) more recent results of extensive studies on the control of the sex ratio in cattle. He says: "Some earlier statistics appeared to indicate that there was a possibility of influencing the sex ratio by paying attention to this point. It was believed to be of such extreme importance as to justify the careful study of the matter on the basis of much more extended statistics. These statistics we have now collected and analyzed and shall publish as soon as possible. In the meantime it may be reported that, with the more extended statistics in hand, it appears to be conclusively established that there is no definite or permanent relation between the time in the heat period at which the cow is served and the sex of the offspring. The apparent relation between these two factors, which is believed by many breeders to exist and which our earlier statistics appeared to indicate, seems now to be purely accidental, and to have arisen only because of the comparative meagerness of the statistics on which the matter was discussed.

Table II.

Showing the Sex of the Calves following Service at Different Parts of the Heat Period.

Heat Period.	Lapsed Time in Hours from Appearance of Heat to Service.	Sex of Offspring.		Per Cent. of Males.
		Males.	Females.	
Early..........	Under 3 hours	200	192	51.0
Middle........	Over 3 and under 8 hours	270	252	51.7
Late...........	Over 8 hours............	187	212	46.9
Totals.........		657	656	50.0

"The summarized results of 1,313 separate and distinct matings given in Table II. will demonstrate this point. In each one of these 1,313 cases the following facts were accurately known, and

reported in such a way that any bias, conscious, or unconscious, of the observer could not have influenced the result: (*a*) the time in hours from the first appearance of heat (œstrum), as noticed by the breeder, to the time the cow was successfully served; (*b*) the sex of the calf resulting from this service.

"It is evident from this table that there is no significant preponderance of females when service is early in heat. There is not now known any method by which the sex ratio or proportion of the sexes in cattle may be effectively controlled by the breeder. A more detailed account of the results, together with further statistics will be published elsewhere."

X. Sex-limited Inheritance in Cattle.

The discovery of the remarkable behavior of certain characters in heredity which can only be plausibly explained by supposing that they are linked with a sex-chromosome or a sex-determining factor still further strengthens our belief in the existence of such a definite factor. Wentworth ('16) reported a case which seems to fall under this general sex-limited group in the inheritance of black-and-white in Ayrshire cattle. While the general breed color is red-and-white, black-and-white animals have been known for some time, as shown by Kuhlman ('15). It is difficult to state whether the black is due to a true black pigment or whether it is simply a very dense red, since chemical solutions have not yet been attempted.

In summarizing the results of the different crosses, Wentworth says, "If the factor of the black-and-white color is represented by B, the hereditary constitutions are as follows: BB is always black-and-white; bb is always red-and-white; Bb is always black-and-white in the male and red-and-white in the female. All of the nine possible matings were discovered, as shown in Table I.

"The expectations here presented are based on the most probable result of each of the matings, considered on an individual basis with reference to the number of animals produced by each type of mating, but without figuring the proportions of the sexes as equal. From these data it would appear that the black-and-white color of Ayrshire cattle behaves in an ordinary

sex-limited manner similar to the horns in sheep as discussed by Wood ('05) and the rudimentary mammæ in swine as reported by Wentworth ('16)."

TABLE I.

RESULTS OF NINE POSSIBLE MATINGS OF AYRSHIRE CATTLE.

Sires.	Dams.	Male Offspring.		Female Offspring.	
		Black-and-white.	Red-and-white.	B'ack-and-white.	Red-and-white.
BB.........	BB......	1	0	3	0
BB.........	Bb......	0	0	0	1
BB.........	bb......	10	0	0	10
Bb.........	BB......	3	0	2	1
Bb.........	Bb......	1	0	1	0
Bb.........	bb......	4	5	0	4
bb.........	BB......	0	0	0	3
bb.........	Bb......	2	1	0	2
bb.........	bb......	0	7	0	9
Total.......	....	21	13	6	30
Expected....	....	20.75	13.25	5.25	30.75

The simple Mendelian scheme of inheritance is quite common in cattle but, to my knowledge, this is the only case of sex-limited inheritance reported in this animal; nevertheless, it is significant, especially in view of our knowledge of the relation between this scheme of inheritance and the sex-chromosomes in many other species.

XI. THE FREE-MARTIN.

The case of the free-martin, the female of two-sexed twins in cattle, is well known to animal breeders to be perfectly sterile although rarely such females are perfectly normal. I do not wish to enter upon a discussion of this subject here for it is a big problem in itself. However, I wish to call the attention of animal husbandmen, who are not in position to keep in touch with all of the zoölogical literature, to the extensive and most painstaking piece of research on the free-martin by Dr. Frank R. Lillie ('17). In this connection I might also call attention to his article on sex-determination and sex-differentiation in mammals (Lillie, '17).

XII. SUMMARY.

1. Thirty-seven chromosomes occur in the spermatogonia. One, the sex-chromosome, is distinctly larger than the others.

2. Nineteen chromosomes appear in the primary spermatocyte division, of which eighteen are bivalent and the other is the unpaired sex-chromosome.

3. In the primary spermatocyte division the heart-shaped sex-chromosome passes undivided to one pole in advance of the other chromosomes.

4. The primary spermatocyte division is evidently the reduction division, giving rise to two different types of secondary spermatocytes, one with the sex-chromosome and the other lacking it.

5. The one type of secondary spermatocyte, which contains the sex-chromosome, gives rise to two spermatids, each containing the sex-chromosome and eighteen ordinary chromosomes.

6. The other type of secondary spermatocyte, which lacks the sex-chromosome, gives rise to two spermatids, each containing only the eighteen ordinary chromosomes.

7. The mature spermatozoa are of two types, equal in numbers. The one type is larger and contains the sex-chromosome. The smaller type is without the sex-chromosome. The larger type is female producing, while the smaller is male producing.

8. Thirty-eight chromosomes occur in the oögonia; two of these are the sex-chromosomes.

9. The reduced number of chromosomes in the female is, in all probability, eighteen ordinary chromosomes and one sex-chromosome which apparently occurs in all of the mature ova.

10. The somatic cells of the male contain thirty-six ordinary chromosomes and one sex-chromosome.

11. The somatic cells of the female contain thirty-six ordinary chromosomes and two sex-chromosomes.

12. The number of chromosomes in the somatic cells of the two sexes is in exact accord with expectations.

13. There is no relation between the time in the heat period at which the cow is served and the sex of the offspring.

14. Sex in cattle, for the present, remains a matter beyond the control of the breeder. It is determined by the sex-chromosomes; it is a matter of inheritance.

15. Sex-limited inheritance strengthens the belief in the chromosome theory of sex-determination.

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EXPLANATION OF PLATES.

PLATE I.

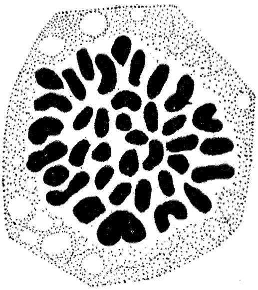
(All of the drawings were made with the aid of a camera lucida, × 2,400, except Figs. 39 and 40 which are × 2,200.)

FIG. 1. Polar view of the metaphase of division in a spermatogonial cell showing thirty-six ordinary chromosomes and the single heart-shaped sex-chromosome.

FIG. 2. Side view of the metaphase of division in a spermatogonial cell showing the thirty-six ordinary chromosomes, and the sex-chromosome at the left.

FIGS. 3 AND 4. Late anaphases of division of spermatogonial cells showing the division of all of the chromosomes, including the large sex-chromosome.

FIGS. 5–9. Late prophase stages of the last spermatogonial cells.



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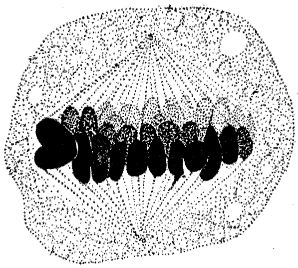

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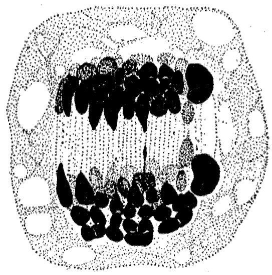

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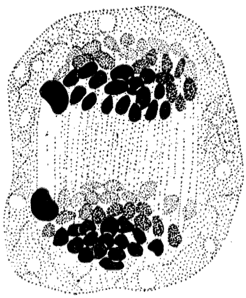

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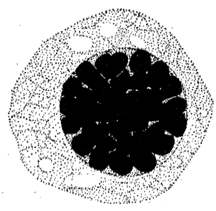

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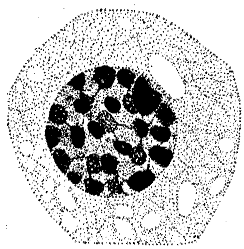

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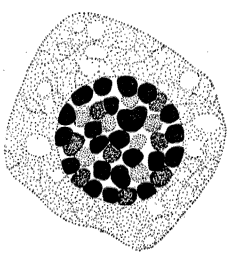

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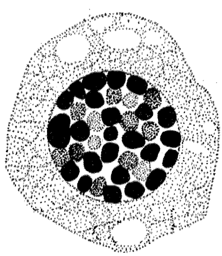

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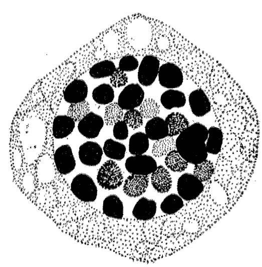

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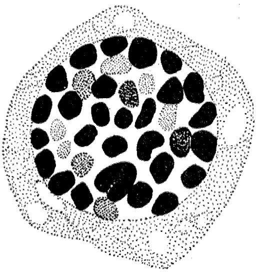
PLATE II.

FIGS. 10 AND 11. Last spermatogonial cells showing thirty-six ordinary chromosomes and the large sex-chromosome.

FIG. 12. Last spermatogonial cell taken from stale tissue showing the persistence of the segregation of the chromosomes in this stage.

FIG. 13. Spireme stage of the primary spermatocyte showing the large sex-chromosome.

FIGS. 14–18. Polar views of metaphase stages of division of the primary spermatocytes showing eighteen bivalent chromosomes and the sex-chromosome which is usually at the periphery of the plate.



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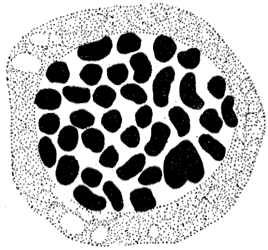

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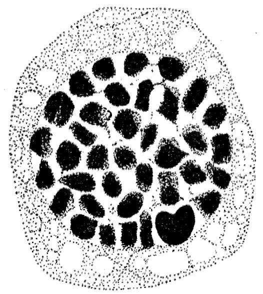

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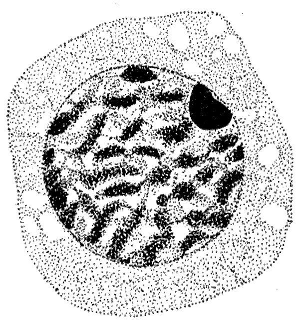

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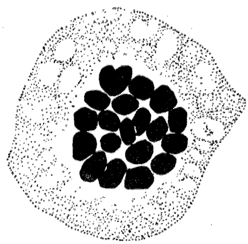

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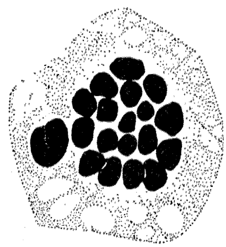

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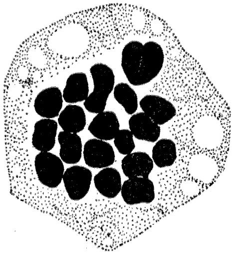

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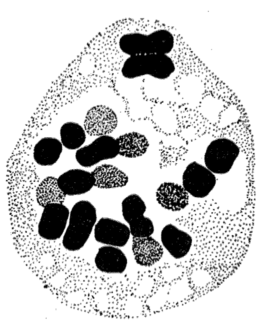

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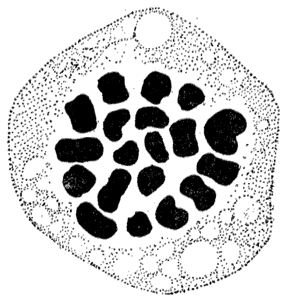

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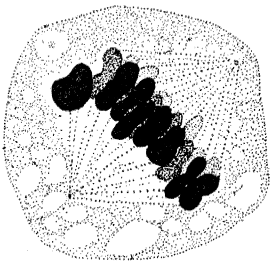
PLATE III.

FIGS. 19–25. Metaphase stages of division of primary spermatocytes showing the passing of the sex-chromosome to one pole in advance of the other chromosomes.

FIGS. 26 AND 27. Late anaphase stages of division of primary spermatocytes showing eighteen chromosomes at one pole and eighteen plus the sex-chromosome at the other.



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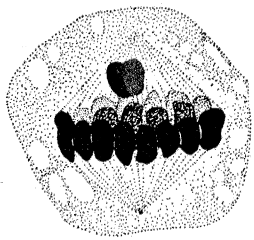

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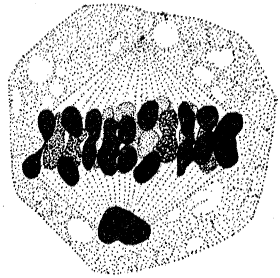

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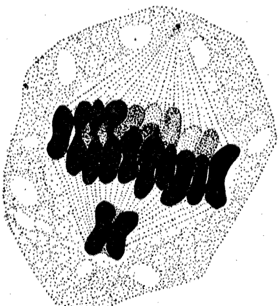

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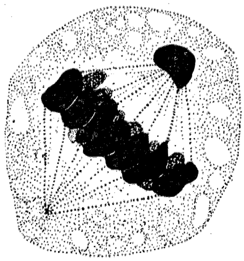

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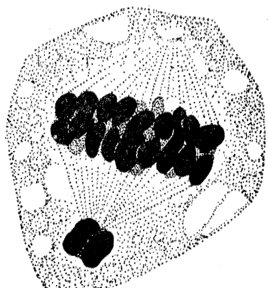

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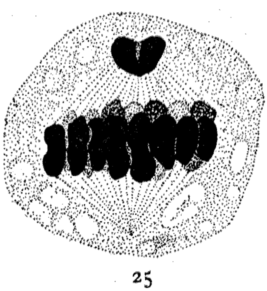

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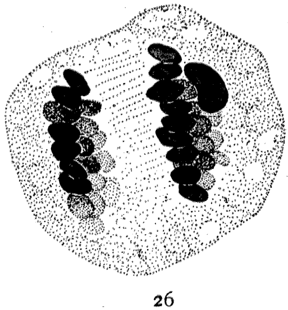

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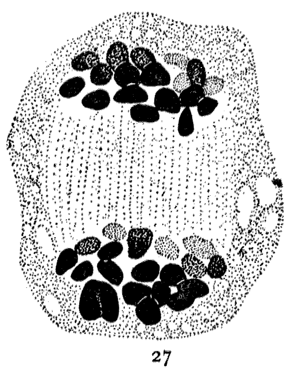

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PLATE IV.

FIGS. 28 AND 29. Cells showing the formation of the secondary spermatocytes from the primary spermatocyte division. The eighteen chromosomes pair loosely to form nine bivalents which are present in the one type of secondary spermatocyte and in the other type are shown the nine bivalents and the sex-chromosome.

FIG. 30. Metaphase stage of division of the one type of secondary spermatocyte showing the nine bivalent chromosomes and the sex-chromosome.

FIG. 31. Metaphase stage of division of the other type of secondary spermatocyte showing only the nine bivalent chromosomes.

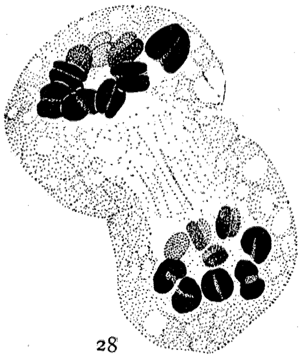
FIGS. 32–34. Division stages of the secondary spermatocyte with the sex-chromosome showing the division of all of the chromosomes, including the sex-element, giving rise to spermatids both of which contain the sex-chromosome. When the division of the cell is complete (Fig. 34) each of the nine ordinary chromosome splits into two so that in reality there are eighteen ordinary chromosomes plus the sex-chromosome in this type of spermatid.

FIGS. 35–37. Division stages of the secondary spermatocyte without the sex-chromosome. Figure 37 shows the splitting up of the nine chromosomes at the poles so that there are in reality eighteen chromosomes passed on to the other type of spermatid.

FIG. 38. A newly formed spermatid showing the eighteen chromosomes.

FIG. 39. A mature spermatozoan of the smaller type undoubtedly without the sex-chromosome.

FIG. 40. A mature spermatozoan of the larger type which undoubtedly contains the sex-chromosome.



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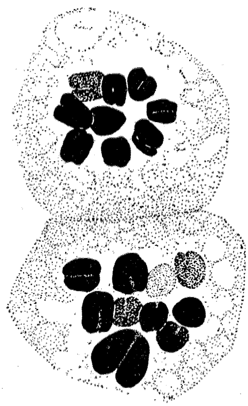

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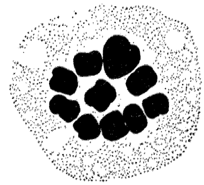

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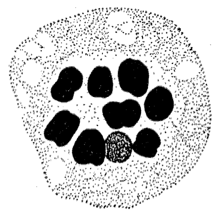

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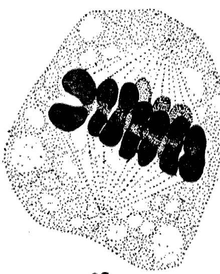

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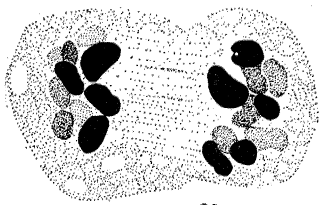

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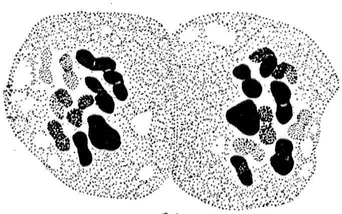

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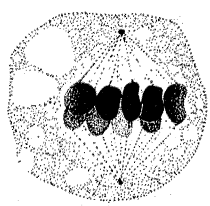

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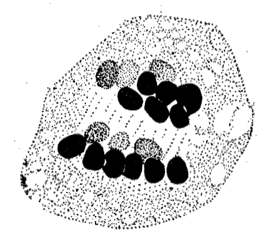

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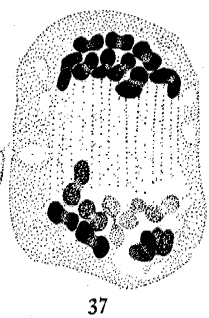

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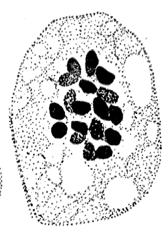

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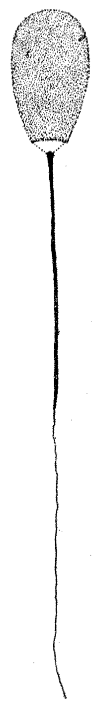

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PLATE V.

FIG. 41. Resting stage of an oögonial cell showing two large nucleoli which are undoubtedly the sex-chromosomes.

FIG. 42. Spireme stage of an oögonial cell showing the two large sex-chromosomes.

FIG. 43. An oögonial cell showing thirty-six newly formed chromosomes and the two large sex-chromosomes.

FIGS. 44 AND 45. Late prophase stages of oögonial cells showing thirty-six ordinary chromosomes and the two sex-chromosomes.

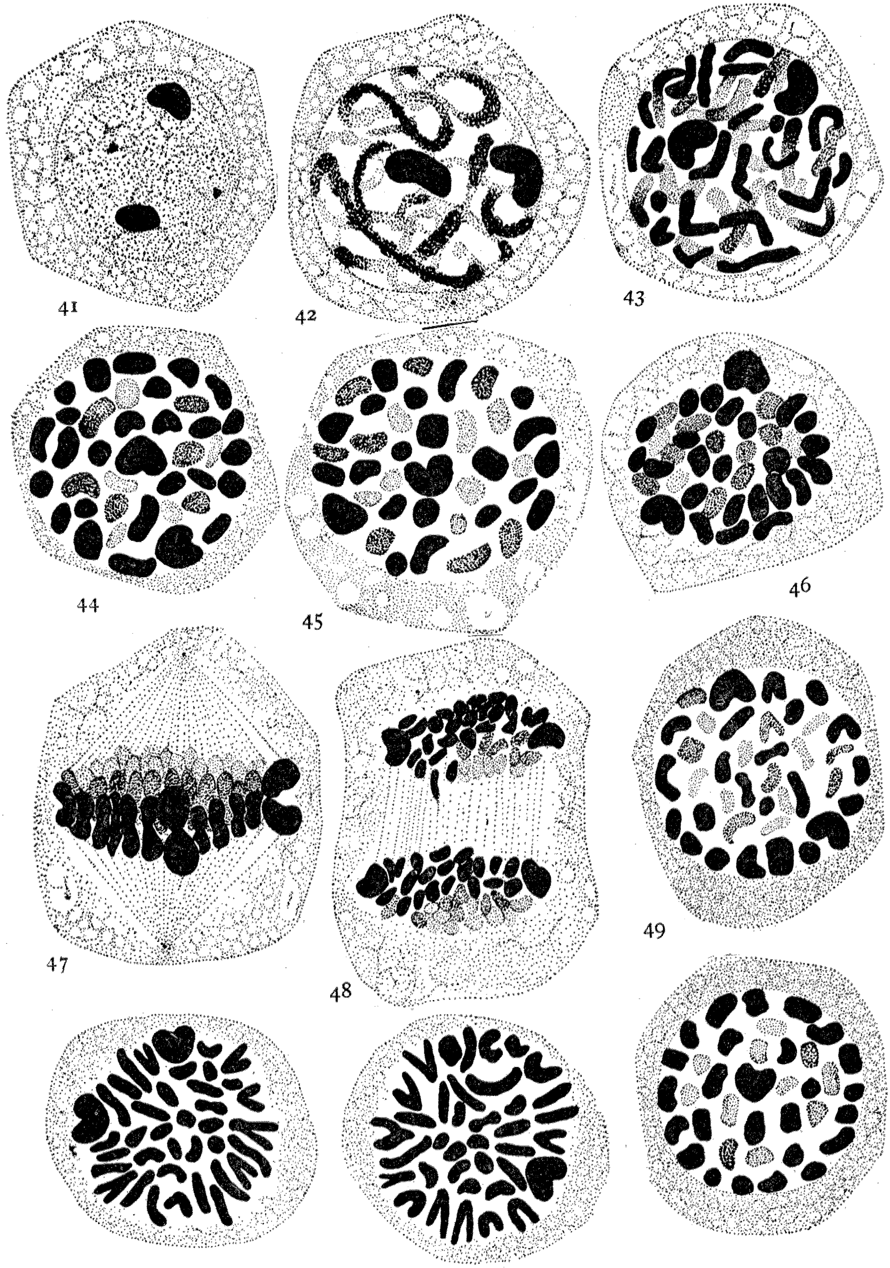
FIGS. 46 AND 47. Metaphase stages of division of the oögonia showing the two sex-chromosomes at the periphery of the plate.

FIG. 49. Liver cell in late prophase stage taken from a female foetus showing thirty-six ordinary chromosomes and the two sex-hromosomes.

FIG. 50. Brain cell in metaphase stage of division taken from a female embryo showing thirty-six ordinary chromosomes and the two sex-chromosomes.

FIG. 51. Brain cell in metaphase stage of division taken from a male embryo showing thirty-six ordinary chromosomes and only one sex-chromosome.

FIG. 52. Liver cell in late prophase stage taken from a male foetus showing thirty-six ordinary chromosomes and only one sex-chromosome.



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